Practical clinical chemistry for medical students

Renal function test (RFT)

Principal :- chemical tests of renal dysfunction includes :

1.Routine testes:

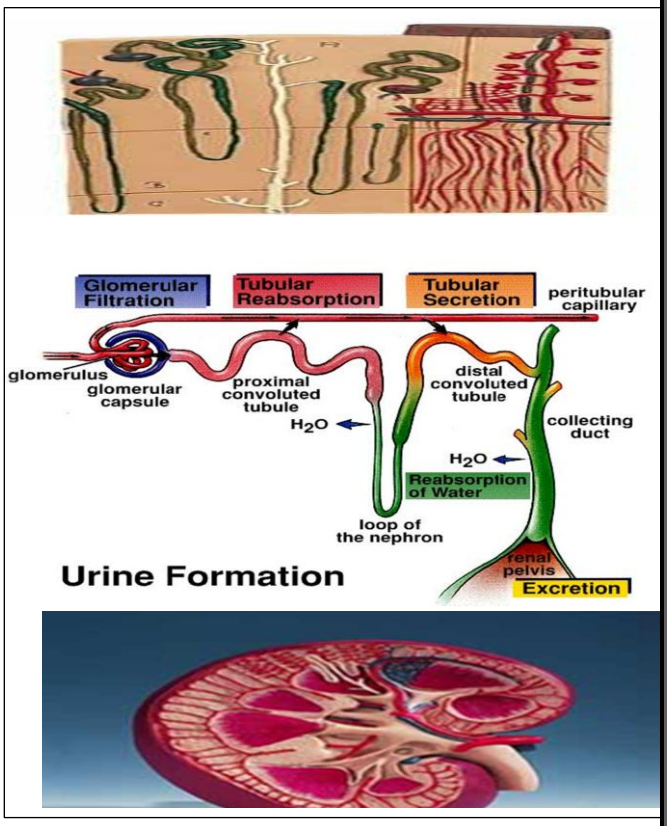
a. Serum

i.S.urea.

ii.S.creatinine.

b. Urine analysis.

c. Creatinine clearance (Ccr)



2.Additional tests :

A.Serum :

i.S.uric acid.

ii.S.phosphate

iii.S.ca, Bicarbonate.

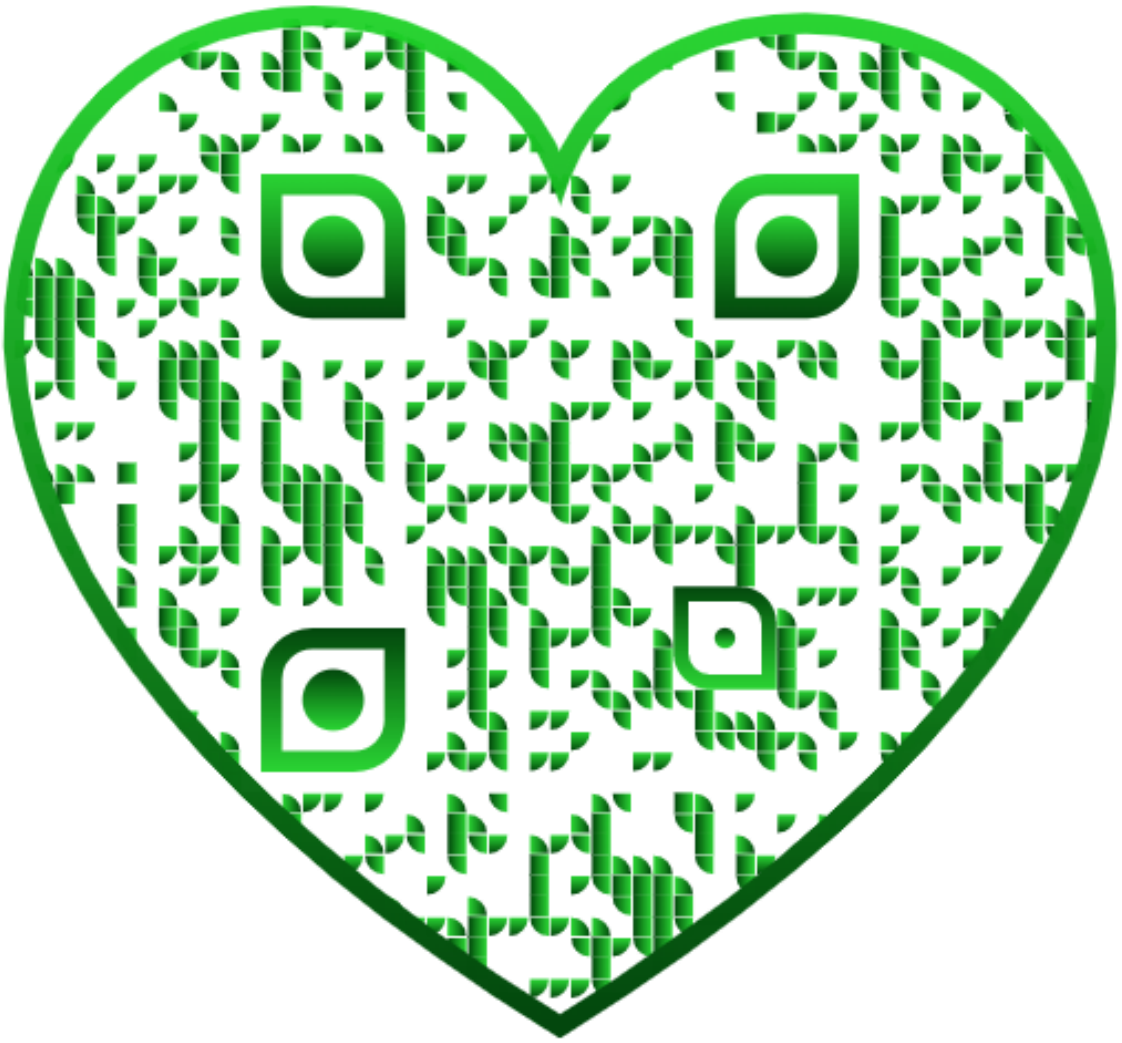
iv.PH, potassium

B.urine:

i. sp- Gravity

ii. Urea conc.

iii.Na.



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1-serum creatinine test :

Clinical application: serum creatinine level is a better indicator of renal function than s. urea or uric acid because it characteristic by :

1.No reabsorbed by renal tubules .

2.Its production rate is not influenced by protein catabolism or other external factors.

3.Its excretion rate is relative constant / day.

***Note** :about 50 % of kidney function most be lost before arise in the S.creatinine conc.

Measurement method :chemical-kinetic method (fixed time)

principle:

S. creatinine + alkaline picrate solution→ red complex.

*the rate at which the red complex develops is measured for a short interval to avoid interference from other serum constituents (e.g. ketones, glucose ,ascorbic acid)

Reagent:

R1:picric acid

R2:NaOH

R3:creatinin stander 2mg/dl

Preparation of W.R: mix equal volume from R1 and R2

Specimen :serum .stable for ……..

Procedure:

Procedure	St.	B	T
W.R	…….ml	……..ml	……ml
Standard.	…… µL	-	-
Specimen	-	-	….. µL

Mix and immediately insert cuvet into the spectrophotometer

Measure absorbance after 30sec.(A1) then after 60 Sec. (A2)

-Zero adjustment: by air, W.R or D.w.

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Calculation of result:

S.creatinine = (A2-A1) test x st.con/ (A2-A1) standard.

.S.I Unit: S. Creatinine [µmol/l] = mg/dl x 10. / m.w. (113) x 1000

Or : mg/dl x 88.4

Adult male : 0.7 - 1.4 mg/dl

Female : 0.6-1.3 mg/dl

Children : 0.4-1.2 mg/dl

Linearity: up to …….mg/dl, if the value > …….. mg/dl, dilute the sample result by ……..

False increased results: Ascorbic acid, Ketone, diet high in meat.

False decreased results: Bilirubin, histidine.

Interpretation of Results:

Increase s.creatinine: its causes are:

a. Impaired renal function.
b. Chronic nephritis.
c. Urinary tract obstructive.
d. Muscle disease.
e. Heart failure, shock, dehydration, Rhabdomyolysis.

Note: Decrease S.creatinine is not clinical significant.

***Panic value of S.creatinine =10mg/gl**

Reporting of results:

S.Creatinine = ……. µmol/l (normal value…… µmol/l).

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2- Creatinine Clearance (Ccr):

Clinical importance:

- most sensitive test to assessing renal function.
- Measure the glomerular filtration rate (GFR) /min.

Specimen:

a- 24hrs Urine.

b- Blood specimen.

Procedure:

Calculate the total volume (TV.) of urine with ml/min. as:

TV-urine ml/min = (T.V. of urine ml/24hrs) ÷ (60x24).

Analysis and calculate the creatinine concentration in both **serum** (S.Creatinine) and **urine** (U. Creatinine) with mg/dl.

Calculate the creatinine clearance (Ccr):

Ccr (ml/min) = U.Creatinine / S.Creatinine x TV urine ml/min.

*URIN specimen is diluted before measure 1/20 . multiply the result by 20.

Normal Value of Ccr:

- Male: 105±20 ml/min.
- Female: 97 ± 30 ml/min.

GFR = 120 m l/min.

E.g.: TV. Of urine = ……. ml/24hrs. s. creatinine = ……. mg/dl.

urine creatinine = ….. mg/dl.

TV. Of urine ml/min = TV of urine ml/24hrs/60 x 24 =……

…….ml/min

Ccr ml/min = U.Creatinine (mg/dl)/S.Creatinine (mg/dl) x TV of urine ml/min

panic value of Ccr < 9ml/min.

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Correction of Ccr. According To Surface Area of Patient:

Ccr. (correct) = Ccr. ml/min. x 1.73/A

A= is body surface area in cubic meter.

1.73 = standard body surface area.

W= weight of patient in Kg.

H=height of patient in cm.

Log A=0.425 log w+0.725 log H-2.144

Interpretation of Ccr Value:

Increase Ccr value: No medical significant.

Decrease value:

b. Decrease GFR: due to Glomerulus's damage or Reduced renal blood flow.

c. Acute tubular damage.

False decreased in Ccr value:

- An error in the specimen collection or
- An error in the creatinine analysis. (S. creatinine is normal)

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3- Serum Urea (Blood Urea Nitrogen {Bun}

Urea nitrogen is the waste product of protein metabolism which synthesis in the liver and excreted by the kidney.

Each 60 mg of urea, contain 28 mg nitrogen.

BUN = (S. urea mg/dl x 28)/ 60 or (BUN = S.urea x 0.467 &

S.urea =BUN x 2.14)

Measurement Method:

Enzymatic method : (Berthelot – urease reaction) is highly specific.

Chemical method: (diacetyl monoxime method): less suitable as a manual method because the color is not stable and the timing is critical. And need to heating at 100 c°

Enzymatic Colorimetric End Point Method (Urease Reaction):

Principle:

Urea + H_2O ^{urease} → ammonia+Co_2

Ammonia + phenol + alkaline hypochlor $^{Na\ nitroprussid}$ → indophenols dye (blue) → read at ……nm.

Specimen: Serum, or EDTA plasma. (Urine dilute 1:100 with N.S)

Reagent: W.R…….

-Urea standard: …….. mg/dl.

Preparation of W.R.:…… ……

Solution is stable ……. at 2-8 c°, avoid exposed to direct light.

Procedure:

Procedure	B	St.	T
W.R	…….ml	……ml	……ml
Standard.	-	…..µL	-
Specimen	-	-	…….µL
Mix → incubate at 37°C for …. min or at room temperature (R.T.) for ……..min. read absorbance (A) of test (T) and standard (S. t.) at			

(…….. nm) (……- ……. nm) against blank (B).

Calculation of result:

s.urea = A.T/A.ST x ST. con. {……. mg/dl}

= ……/…….. x …….=…….

{BUN= …….. mg/dl}

.**S.I Unit:** S.urea [mmol/l] = mg/dl x 10 /m.w. (60).

OR:mmol/l=mg/dl x0.1665

S.urea [mmol/l] =**……**

Reporting Of Results:

S. urea = …… mmol/L (NR=……mmol/L).

Linearity: Up to ……mg/dl, if the value >……mg/dl, dilute the sample as 1:…….. with Nac1 0.9% and repeat the test. Multiply the final result by……...

Interpretation of Results:

Increase s.urea: its causes are:

2. Increase protein diet. Or catabolism.
3. Drugs e.g. cortisol, stress.
4. Renal disease (pre, renal, post renal).

Decrease s.urea:

1. Late pregnancy.
2. Starvation & Grossly protein deficiency.
3. Sever liver disease.

Panic value of BUN: > 100 mg/dl. (S. Urea > 214 mg/dl)

Investigation of Renal Dysfunction:

1. BUN.
2. S.Creatinine.
3. Ccr.
4. BUN/S. Creatinine ration.

a. Ratio: 10-15 indicate intrinsic renal dysfunction.
b. Ration: > 15 indicate pre renal or urinary tract obstruction.
c. Ration: < 10 indicate:
    i. Decrease in S. Urea.
    ii. Acute tubular necrosis.
    iii. Dialysis.
    iv. Increased in S.Creatinine.

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4-Serum Uric Acid (S.U.A)

Uric acid is a purin compound circulates in plasma as Na-urate and excreted by kidney. It's derived from the breakdown of nucleic acid.

Indication for test:

1. Diagnosis and treatment of gout disease.
2. Indicator of a large-scale breakdown of nucleic acid such as in: Toxemia, massive irradiation of tumors. Leukemia, polycythemia, resolving pneumonia
3. Renal disease as confirmatory check test.

Measurement Methods

Two methods for S.U.A determination:

Classical chemical method: (phosphoTungstic acid) not specific.

Enzymatic method: (Uricase reaction) : specific for urate

Enzymatic Method (Uricase Reaction)

Principle

Uric acid + O_2 + $_2$ H_2O ^{uricase} → Allantion + CO_2 + H_2O_2

2 H_2O_2 + 4aminoantipyrine + DHBS. ^{peroxidase} → chromogen + 4H_2O

Read absorbance at ……nm.

* DHBS= dichloro – 2hydroxy benzene sulfonic acid

Specimen: Serum and free from hemolysis .stable for ……

Procedure:

*	B	St.	T
W.R	……ml	…..ml	……ml
Standard.	-	…..µL	-
Specimen	-	-	…..µL
Mix → incubate at 37°C for ….min or at room temperature (R.T.) for … min. read absorbance (A) of test (T) and standard (S. t.) at (….. nm) (….- …. nm)			

against blank (B).The color stable for …….

- **Calculation:**

S.U.A. = A. T / A. St. x ST. CON. =**……**

S.I Unit: S.U.A [µmol/1] = mg/dl x 10 m.w. (168) x 1000= **……**

OR: S.U.A [µmol/1] = mg/dl x59.5

Normal Value: Serum or plasma: 2.5-7 mg /dl.

Reporting of result :-

S.U.A = ……. µmol/1 (……µmol/1)

- **Linearity :** up to …….. mg /dl. if the value > …….mg /dl, dilute the sample as 1:….with NaCL 0.9% and repeat the test. Multiply the final result by ……..

False increased result: Turbid serum.

False decreased result: -

Ascorbic acid, Bilirubin.

Bacterial contamination of specimen.

In Lipemic sample the serum blank must be run as:

(…….. µL sample +…….ml D. w. read and record of blank absorbance and subtract reading from the test absorbance).

Interpretation of Results:

Increase S.Uric acid:

1. Primary gout (idiopathic), dietary purines (organic meats e. t.c.)
2. Leukemia, polycythemia, toxemia of pregnancy, resolving pneumonia, irradiation of carcinomas, sickle cells.
3. Alcohol ingestion, lactic acidosis, Thiazide diuretics, ketoacidosis (diabetes, starvation).
4. Renal disease.

Decrease S.uric acid:

1. ACTH, cortisol, estrogen, aspirin, penicillin
2. Allopurinol.